



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/GB92/00498 <b>(22) International Filing Date:</b> 19 March 1992 (19.03.92)  <b>(30) Priority data:</b> 9106177.0                      22 March 1991 (22.03.91)                      GB  <b>(71) Applicant (for all designated States except US):</b> ASTON MOLECULES LIMITED [GB/GB]; 10 Holt Court South, Aston Science Park, Birmingham B7 4EJ (GB).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only) :</b> RATHBONE, Daniel, Lee [GB/GB]; 78 Newcombe Road, Earlsdon, Coventry CV5 6NL (GB). SLACK, John, Alfred [GB/GB]; 55 Woodford Green Road, Hall Green, Birmingham B28 8PH (GB). GRIFFIN, Roger, John [GB/GB]; 6 St Leonard's Walk, Lancaster Park, Morpeth, Northumberland NE61 3SZ (GB). QUARTERMAN, Charmaine, Paulina [GB/GB]; 56 Hollowfields Close, Southcrest, Redditch B98 7NR (GB).		<b>(74) Agent:</b> H.N. & W.S. SKERRETT; Charles House, 148/9 Great Charles Street, Birmingham B3 3HT (GB).  <b>(81) Designated States:</b> AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), SE (European patent), US.  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> SUBSTITUTED DIPHENYLETHYLENES AND ANALOGUES OR DERIVATIVES THEREOF		
<b>(57) Abstract</b>  Substituted diphenylethylenes such as the compound known as Combretastatin A4 ( <i>cis</i> -1-(3-Hydroxy-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene) and analogues or derivatives thereof are synthesised in a convergent process wherein the ylide form of a triphenyl phosphonium salt of a trialkoxy benzyl halide is reacted in a Wittig coupling reaction with a benzaldehyde compound having a protected hydroxyl or O-phosphate group in the 3-position and an alkoxy group in the 4-position. Combretastatin A4 analogues and derivatives having improved or enhanced aqueous solubility and photostability characteristics are disclosed suitable for use as biodegradable pro-drugs in pharmaceutical formulations for clinical use.		

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SUBSTITUTED DIPHENYLETHYLENES AND ANALOGUES OR  
DERIVATIVES THEREOF

This invention relates to substituted diphenyl-  
5 ethylenes of the kind exemplified by the compound known  
as Combretastatin A4 (*cis*-1-(3-Hydroxy-4-methoxyphenyl)-  
2-(3,4,5-trimethoxyphenyl)ethene) and analogues or  
derivatives thereof.

10 BACKGROUND

Combretastatin A4 in particular is known from the  
work of G.R. Pettit et al. (see, for example, Pettit,  
G.R., et al. Experientia, 1989, 45, 209-211), and has  
15 been shown to be active *in vitro* as an inhibitor of  
tubulin polymerisation and, additionally, to inhibit the  
growth of murine lymphocytic leukemia. It is accordingly  
of interest as a promising therapeutic agent for use in  
chemotherapy, especially as an anti-neoplastic or anti-  
20 cancer agent, but unfortunately it has been found to have  
limited solubility in pharmaceutically acceptable  
solvents and this characteristic has delayed its entry  
into Phase I clinical trials. Also, it has not been easy  
to synthesise efficiently.

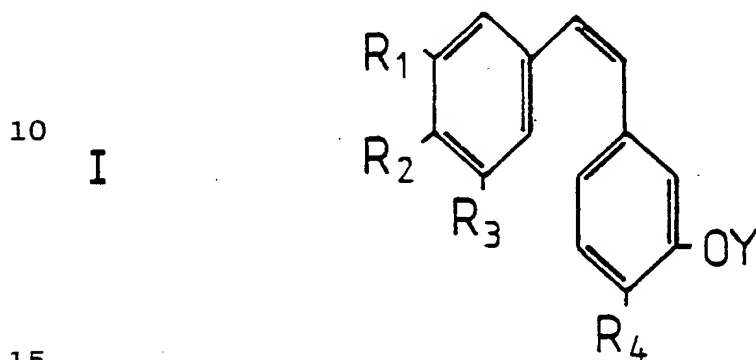
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SUMMARY OF THE INVENTION

The present invention has developed from efforts to  
devise an improved process for synthesizing  
30 Combretastatin A4 and from efforts to produce analogues  
or derivatives having greater aqueous solubility, more  
suitable for use in pharmaceutical formulations, and  
capable of acting as pro-drugs which can be biologically  
degraded or broken down to release the active  
35 Combretastatin A4 component within the body after being  
administered to a patient in need of treatment. Such  
pro-drugs generally exhibit greater stability to

oxidative decomposition than the parent drug.

More specifically, in one aspect the invention provides a process for preparing a substituted diphenyl-  
5 ethylene compound having the general structure



wherein  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are alkoxy groups, and Y is hydrogen, a phosphate or phosphate derivative, an amino acid carbamate derivative, or a derivative of a  
20 carbohydrate or polyhydroxylated compound, the process being characterised in that it is a convergent process that includes the step of reacting, in a Wittig coupling reaction, an ylide triphenyl phosphonium salt of a trialkoxy benzyl halide with a benzaldehyde compound:



wherein X is a protective group.

35

In preferred embodiments X comprises a silyl group or phosphate ester group. Also, the alkoxy groups

generally each contain 1-6 carbon atoms, and are most preferably methoxy or ethoxy.

The process of the invention also provides a number  
5 of novel intermediate compounds.

The invention further provides novel analogues or derivatives of Combretastatin A4 (*cis* isomer of Compound I wherein Y is hydrogen) which have enhanced aqueous  
10 solubility and photostability and which are especially suitable for use as pro-drugs in pharmaceutical formulations for clinical use.

The invention also includes pharmaceutical  
15 formulations comprising or containing such novel analogues or derivatives made up for administration in any suitable manner, for example parentally (including intravenously, intramuscularly and subcutaneously) or orally. Such formulations containing or incorporating  
20 therapeutically effective non-toxic amounts, conveniently in unit dosage form, of the active drug compound, together possibly with at least one other ingredient providing a compatible pharmaceutically acceptable additive, diluent or excipient, may be prepared by any of  
25 the methods well known in the art of pharmacy.

Particularly preferred novel analogues of Combretastatin A4 are phosphate derivatives and salts thereof which have enhanced aqueous solubility and which  
30 are susceptible to enzymic dephosphorylation so that they can act as pro-drugs of Combretastatin A4. Other, alternative, novel analogues that may also serve as satisfactory biodegradable pro-drugs comprise amino-acid carbamate derivatives, e.g. glycine carbamate  
35 derivatives, and carbohydrate or polyhydroxylated derivatives.

MORE DETAILED DESCRIPTION

The invention will be further described and exemplified with specific reference to the preparation of  
5 Combretastatin A4 and Combretastatin A4 analogues, particularly Combretastatin A4 phosphate (including salts thereof) and Combretastatin A4 glycine carbamate derivatives.

10 Combretastatin A4 phosphate has been shown to be stable in aqueous solution and to degrade *in vitro* to Combretastatin A4 when incubated with either acid phosphatase or alkaline phosphatase.

15 First, there is presented below the analytical conditions that were used to demonstrate that the Combretastatin A4 phosphate has the desired properties for formulation for clinical trial. Then, there are presented the synthetic details for specific analogues of  
20 Combretastatin A4 herein mentioned.

Analytical Methodology - Non Biological samples

Combretastatin A4 phosphate can be determined by HPLC using the following chromatographic conditions:

25

Column: Lichrosorb (Regd. Trade Mark)

RP Select B. 125 x 4mm.

Mobile phase: Methanol : 0.5% acetic acid (50:50)

Flow rate: 2 ml/min

30

Detection: UV at 240nm

Injection volume: 0.02ml

Analysis time: 15 minutes

Approximate retention time: 6 minutes

35

Using the same chromatographic conditions Combretastatin A4 has a retention time of approximately 11 minutes.

### Aqueous Solubility

The solubility of the diammonium salt of Combretastatin A4 phosphate has been determined by visualisation. By progressively adding small known amounts of the material to 2ml water the solubility of this salt was found to be approximately 2.8mg/ml.

### Aqueous Stability

A solution of Combretastatin A4 Phosphate diammonium salt (approximately 0.14mg/ml) was protected from light and stored at room temperature. Aliquots were taken over a 6 hour period and analysed by HPLC in order to determine stability. The A4 phosphate was shown to be stable over this period.

### Stability to Human Plasma

In a preliminary investigation the stability of Combretastatin A4 phosphate has been assessed in "pooled" plasma derived from two healthy human subjects. Freshly prepared plasma was incubated at 37°C and aqueous Combretastatin A4 phosphate added to produce a starting concentration of approximately 28µg/ml. Aliquots of plasma were taken for HPLC analysis at zero time and at intervals thereafter.

Stability was monitored indirectly by HPLC determination of the Combretastatin A4 formed. Plasma was acidified with 0.1N HCl and the Combretastatin A4 extracted with ethyl acetate. The organic layer was evaporated to dryness under vacuum at ambient temperature and the residue reconstituted in methanol:water (50:50). The chromatographic conditions were as hereinbefore described.

No degradation of Combretastatin A4 phosphate was obtained over a 45 minute period. However, when calf

intestine alkaline phosphatase was added to the incubate Combretastatin A4 phosphate underwent degradation to form Combretastatin A4.

5 Action of Alkaline Phosphatase

Combretastatin A4 phosphate was incubated at 37°C in 10mM tris buffer, pH 8. In the presence of calf intestinal phosphatase the A4 phosphate was dephosphorylated to produce Combretastatin A4. In contrast, Combretastatin A4 phosphate was stable in a control incubation containing no enzyme.

Synthesis of Combretastatin A4 and Combretastatin A4 Phosphate

15 In an example of the process of the present invention, using a convergent five-step synthesis Combretastatin A4 has been prepared with an overall yield of 27.8% (this may be compared with the linear six-step synthesis published by Pettit et al [see Pettit, G.R., et al. Experientia, 1989, 45, 209-211, Pettit, G.R., et al. J. Org. Chem., 1985, 50, 3404-3406 and Pettit, G.R., et al. J. Nat. Products, 1987, 50, 119-131] having an overall yield of 18.9%).

25 The synthesis briefly was as follows:

3,4,5-Trimethoxybenzyl alcohol was brominated to give 3,4,5-trimethoxybenzyl bromide in 86% yield. This was converted immediately into the corresponding triphenylphosphonium salt in 84% yield. 3-Hydroxy-4-methoxybenzaldehyde was silylated in 92% yield to give the protected form 4-methoxy-3-(hexyldimethylsilyloxy)benzaldehyde. This was coupled in a Wittig coupling reaction to the ylide form of the phosphonium salt from above to give a 1/1 mixture of the silylated Combretastatin A4 and its trans isomer. The desired cis isomer was separated by chromatography



in a yield of 47% and deprotected in 82% yield to give Combretastatin A4. This was phosphorylated to give Combretastatin A4 phosphate bis *t*-butyl ester (77%) which was hydrolysed (78%) and isolated as the ammonium salt and then converted to the potassium salt (55%).

The key step is the Wittig coupling of the ylide from 3,4,5-trimethoxybenzylphosphonium bromide and 4-methoxy-3-(*tert*-butyldimethylsilyloxy)benzaldehyde. The latter compound, the product from the Wittig coupling step, the three Combretastatin A4 phosphate derivatives and the glycine carbamate derivative detailed above are all novel compounds so far unreported in the literature.

The following more detailed specific examples and descriptions of synthetic stages in the preparation of particular compounds are now presented by way of further illustration of the invention, but should not be construed in any way as a limitation thereof.

#### EXAMPLE 1

##### Preparation of Combretastatin A4

25

(a) 3,4,5-Trimethoxybenzyl bromide (Compound 1)

Trimethylchlorosilane (28.0ml, 0.221mol) was added to a vigorously stirred suspension of lithium bromide (15.6g, 0.180mol) in dry acetonitrile (200ml) under an atmosphere of argon. To this was added 3,4,5-trimethoxybenzyl alcohol (17.82g, 0.090mol) dissolved in dry acetonitrile (15ml). This resulted in a homogeneous solution which was set aside at room temperature for 18 hours by which time a white precipitate had formed. Ethyl acetate (100ml) was added and the solution was washed successively with water (2x150ml), saturated sodium hydrogen carbonate solution (2x50ml) and water

(50ml). The organic layer was dried over magnesium sulphate and evaporated under reduced pressure to give 3,4,5-trimethoxybenzyl bromide as a yellow solid (20.3g, 0.778 mol, 86% yield). This was used immediately without purification in the next stage to prepare the corresponding phosphonium salt.

(b) 3,4,5-Trimethoxybenzyl phosphonium bromide  
(Compound 2)

To a solution of 3,4,5-trimethoxybenzyl bromide (20.2g, 0.774mol) in dry toluene (50ml) was added a solution of triphenylphosphine (22.0g, 0.893mol) in dry toluene (40ml). The solution was stirred at room temperature for 46 hours during which time a white solid formed. The white solid was collected by filtration, washed with toluene (70ml) and dried in vacuo over phosphorous pentoxide to give 3,4,5-trimethoxybenzylphosphonium bromide as a white powder (33.8g, 0.065mol, 84% yield). mp 221-222°C (Lit 222-223°C).

(c) 4-Methoxy-3-(thexyldimethylsilyloxy)benzaldehyde  
(Compound 3)

To an ice-cold stirred solution of 3-hydroxy-4-methoxybenzaldehyde (30.0g, 0.197mol) in dry tetrahydrofuran (250ml) under an atmosphere of argon was added thexyldimethylsilyl chloride (59.3ml, 0.301mol) followed by triethylamine (35.0ml, 0.252mol). The mixture was stirred at room temperature for 3 days during which time a white precipitate formed. The solid was removed by filtration through a pad of celite. The celite was washed with tetrahydrofuran which was added to the filtrate and evaporated under reduced pressure. The residue was chromatographed on silica, eluting with acetone-hexane 20/80. Fractions corresponding to  $R_f$  0.43 were combined and evaporated under reduced pressure. The residual oil was heated at 100°C at 0.7mbar for 6 hours

to remove thexyldimethylsilyl chloride which distilled out. The undistilled remainder comprised 4-methoxy-3-(thexyldimethylsilyloxy)benzaldehyde as a yellow oil (53.47g, 0.182mol, 92% yield).

- 5  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ; ppm  $\delta$  from TMS): 0.16 (s, 6H,  $\text{SiMe}_2$ ); 0.92 (d, 6H,  $J=6.9\text{Hz}$ ,  $\text{CHMe}_2$ ); 0.94 (s, 6H,  $\text{CMe}_2$ ); 1.72 (septet, 1H,  $J=6.9\text{Hz}$ ,  $\text{CHMe}_2$ ); 3.84 (s, 3H, OMe); 6.91 (d, 1H,  $J=8.3\text{Hz}$ , Ar-H5); 7.33 (d, 1H,  $J=2.0\text{Hz}$ , Ar-H2); 7.43 (dd, 1H,  $J=8.3\text{Hz}$ , 2.0Hz, Ar-H6); 9.78 (s, 1H, CHO).
- 10  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ; ppm  $\delta$  from TMS): -2.8 ( $\text{SiMe}_2$ ); 18.4 ( $\text{CHMe}_2$ ); 20.0 ( $\text{SiCMe}_2$ ); 25.2 ( $\text{SiCMe}_2$ ); 33.9 ( $\text{CHMe}_2$ ); 55.4 (OMe); 111.1, 119.9, 126.2 (protonated aromatics); 130.1, 145.3, 156.6 (quaternary aromatics); 190.8 (CHO).
- IR (thin film) 3020, 1725, 1620, 1600, 1540, 1470, 1315, 1160 875  $\text{cm}^{-1}$ .

(d) cis-1-(4-Methoxy-3-(thexyldimethylsilyloxy)phenyl)-2-(3,4,5-trimethoxyphenyl)ethene (Compound 4)

To an ice-cold stirred suspension of 3,4,5-trimethoxybenzylphosphonium bromide (8.20g, 15.7mmol) in dry tetrahydrofuran (200ml) under an atmosphere of argon was added n-butyllithium in hexane (2.0M, 8.0ml, 16.0mmol) to give a dark red mixture which became homogeneous after being stirred at room temperature for 1.5 hours.

4-Methoxy-3-(thexyldimethylsilyloxy) benzaldehyde (5.15g, 17.5mmol) dissolved in dry tetrahydrofuran (10ml) was added and the mixture was stirred in the dark at room temperature for 20 hours. Ice (50ml) was added and the mixture was extracted with diethyl ether (4x80ml). The combined extracts were dried over magnesium sulphate and the solvent was removed under reduced pressure. The residual oil was chromatographed on silica, eluting with, ethyl acetate-petrol (bp 60-80°C) 1/9. Fractions corresponding to  $R_f$  0.21 were combined and the solvent was removed under reduced pressure to yield cis-1-(4-methoxy-3-(thexyldimethylsilyloxy)phenyl)-2-(3,4,5-trimethoxy-

phenyl)ethene as a yellow oil (3.42g, 7.46mmol, 47% yield).

<sup>1</sup>H NMR (CDCl<sub>3</sub>; ppm δ from TMS): 0.06 (s, 6H, SiMe<sub>2</sub>); 0.87 (s, 6H, SiCMe<sub>2</sub>); 0.89 (d, 6H, J=6.8Hz, CHMe<sub>2</sub>); 1.67 (septet), 1H, J=6.8Hz, CHMe<sub>2</sub>); 3.68 (s, 6H, OMe); 3.74 (s, 3H, OMe); 3.81 (s, 3H, OMe); 6.39 (d, 1H, J=12.3Hz, ArCH); 6.45 (d, 1H, J=12.3Hz, ArCH); 6.48 (s, 2H, ArH); 6.71 (d, 1H, J=8.3Hz, ArH); 6.78 (d, 1H, J=1.9Hz, ArH); 6.83 (dd, 1H, J=8.3Hz, 2.0Hz, ArH).

<sup>13</sup>C NMR (CDCl<sub>3</sub>; ppm δ from TMS): -2.9 (SiMe<sub>2</sub>); 18.4 (SiCMe<sub>2</sub>); 20.0 (CHMe<sub>2</sub>); 25.1 (SiCMe<sub>2</sub>); 33.9 (CHMe<sub>2</sub>); 55.4, 55.8, 60.7 (OMe); 105.7, 111.6, 121.2, 122.7 (protonated Ar); 128.6, 129.6 (ArCCAr); 129.9, 133.0, 136.9, 144.9, 150.3, 152.8 (quaternary Ar).

MS (EI) m/e=457 (M-H)<sup>+</sup>.

The *trans*-isomer (R<sub>F</sub> 0.10) could be obtained by further elution.

(e) cis-1-(3-Hydroxy-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (Combretastatin A4) (Compound 5)

To a solution of *cis*-1-(4-methoxy-3-(*tert*-butylmethoxysilyloxy)phenyl)-2-(3,4,5-trimethoxyphenyl)ethene (2.032g, 4.43mmol) dissolved in dry tetrahydrofuran (20ml) under an atmosphere of argon was added tetrabutylammonium fluoride (1.0M in tetrahydrofuran, 4.5ml, 4.5mmol). The resulting strongly yellow solution was set aside in the dark at room temperature for 25 hours. Diethyl ether (150ml) was added and the solution was washed with water (2x50ml). The organic fractions were dried over magnesium sulphate and evaporated under reduced pressure to give a white crystalline solid. The crude product was recrystallised from ethyl acetate-petrol (bp 60-80°C) to give combretastatin A4 as white needles (1.147g, 3.63mmol, 82% yield). mp 114.0-115.2°C

(corrected).

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ ppm from TMS: 3.66 (6H, s, OMe); 3.79 (3H, s, OMe); 3.80 (3H, s, OMe); 5.67 (1H, s, OH); 6.37 (1H, d, J=12.2Hz, ArCH); 6.43 (1H, d, J=12.2Hz, ArCH);  
5 6.50 (2H, s, Ar-H); 6.69 (1H, d, J=8.3Hz, Ar-H); 6.74 (1H, dd, J=8.3Hz, 2.0Hz, Ar-H); 6.89 (1H, d, J=2.0Hz, Ar-H).

<sup>13</sup>C NMR (CDCl<sub>3</sub>) δ ppm from TMS: 55.8 (OMe); 60.8 (OMe); 106.0, 110.3, 115.0, 121.0 (protonated Ar); 130.4, 132.6,  
10 136.5, 145.2, 145.7, 152.7 (quaternary Ar); 128.9, 129.4 (ArCHCHAr).

IR (nujol mull) 3290cm<sup>-1</sup> (OH); 1675cm<sup>-1</sup> (C=C); 1605cm<sup>-1</sup> (Ar); 1420cm<sup>-1</sup> (Ar).

MS (EI) m/e = 316 (M<sup>+</sup>); 301 (M-CH<sub>3</sub>)<sup>+</sup>

15

## EXAMPLE 2

### Preparation of Combretastatin A4 Phosphate potassium salt

20 (a) cis-1-(3-O-Phosphate-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene bis t-butyl ester (Combretastatin A4 phosphate bis t-butyl ester) (Compound 6)

A dry solution of m-chloroperoxybenzoic acid (MCPBA) was prepared in dichloromethane: Commercial 50%  
25 MCPBA (2.30g, nominally 6.6mmol) was dissolved in dichloromethane (20ml) and dried over magnesium sulphate. The mixture was filtered and used as such in the reaction described below.

30 To a solution of cis-1-(3-hydroxy-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (1.03g, 3.26mmol) and di-t-butyl N,N-diethylphosphoramidite (1.30g, 5.22mmol) dissolved in dry tetrahydrofuran (20ml) was added 1H-tetrazole (0.70g, 10.0mmol). The resulting homogeneous  
35 solution was set aside at room temperature under an atmosphere of argon in the dark for 35 minutes. A white precipitate formed. The mixture was cooled to -70°C and

treated with the MCPBA solution. The mixture was stirred at -70°C for 15 minutes and then warmed to room temperature over 10 minutes before being quenched with a 10% aqueous solution of sodium sulphite (25ml). The mixture was stirred for 10 minutes and then extracted with dichloromethane (3x30ml). The dichloromethane portions were combined and washed with saturated aqueous sodium hydrogen carbonate (20ml). The aqueous layer was back-extracted with dichloromethane (30ml). The dichloromethane portions were combined, dried over magnesium sulphate and evaporated under reduced pressure. The residue was chromatographed on silica, eluting with acetone-petrol (bp 60-80°C) 3/7. Fractions corresponding to  $R_F$  0.2 were combined and evaporated under reduced pressure to give combretastatin A4 phosphate bis t-butyl ester as a yellow oil (2.03g, 3.99mmol, 77% yield).  $^1H$  NMR ( $CDCl_3$ ; ppm  $\delta$  from TMS): 1.37 (s, 9H,  $CMe_3$ ); 3.59 (s, 6H, OMe); 3.72, 3.73 (s, 3H, OMe); 6.32 (d, 1H,  $J=12.2Hz$ , ArCH); 6.37 (d, 1H,  $J=12.2Hz$ , ArCH); 6.41 (s, 2H, ArH); 6.70 (d, 1H,  $J=8.5Hz$ , ArH); 6.93 (d, 1H,  $J=8.4Hz$ , ArH); 7.23 (s, 1H, ArH).  $^{13}C$  NMR ( $CDCl_3$ )  $\delta$  ppm from TMS: 29.6 (d,  $J=4.3Hz$ ,  $CMe_3$ ); 35.7, 55.8, 60.8 (OMe); 83.4 (d,  $J=7.9Hz$ ,  $CMe_3$ ); 105.8, 112.0 (Ar), 121.66 (d,  $J=2.9Hz$ , Ar), 125.6 (Ar); 128.9, 129.1 (ArCCAr); 129.7, 132.5, 136.9 (Ar); 140.2 (d,  $J=7.3Hz$ , Ar); 149.7 (d,  $J=6.2Hz$ , Ar); 152.8 (Ar).  $^{31}P$  NMR ( $CDCl_3$ )  $\delta$  ppm from phosphoric acid -14.3 IR (thin film)  $2970cm^{-1}$  (Ar);  $1570cm^{-1}$  (C=C);  $1505cm^{-1}$  (Ar);  $1280cm^{-1}$  (phosphate).

(b) cis-1-(3-O-Phosphate-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene ammonium salt (Combretastatin A4 phosphate ammonium salt) (Compound 7)

To an ice cold solution of cis-1-(3-O-phosphate-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene bis t-butyl ester (0.168g, 0.33mmol) in dry dichloromethane (5ml) was added a solution of trifluoroacetic acid

(0.25ml, 3.24mmol) dissolved in dry dichloromethane (5ml). The solution was set aside in the dark at ice bath temperature for 30 minutes and then at room temperature for 2.5 hours. The solvents were evaporated  
 5 under reduced pressure and the residue was evaporated again with ethanol (2x10ml). Concentrated aqueous ammonia solution (6 drops) and ethanol (3ml) was added. A white crystalline solid separated immediately which was collected by filtration, washed with ethanol and dried in  
 10 vacuo to give Combretastatin A4 phosphate ammonium salt (0.110g, 0.26mmol, 78% yield). mp 208.4-209.2°C.

<sup>1</sup>H NMR (D<sub>2</sub>O) ppm referenced to δ(HOD)=4.80ppm: 3.67 (6H, s, OMe[Ar-1]); 3.74 (3H, s, OMe [Ar-1]); 3.81 (3H, s, OMe [Ar-2]); 6.52 (1H, d, J=12Hz, ArCHCHAR); 6.62 (1H, d,  
 15 J=12Hz, ArCHCHAR); 6.64 (2H, s, Ar-H); 6.90 (1H, m, Ar-H); 6.97 (1H, m, Ar-H); 7.25 (1H, m, Ar-H).

IR (nujol mull) 1575 cm<sup>-1</sup> (C=C); 1515 cm<sup>-1</sup> (Ar); 1465cm<sup>-1</sup> (Ar); 1245cm<sup>-1</sup> (phosphate).

MS (FAB) m/e=396 (M-2NH<sub>3</sub>)<sup>+</sup>

20

#### Elemental analysis

		Required (%)		Found (%)
		mono-	bis-	
		ammonium salt		
25	C	52.30	50.23	50.99
	H	5.81	6.28	5.95
	N	3.39	6.51	4.48
	P	7.51	7.21	7.20

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(c) cis-1-(3-O-Phosphate-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene potassium salt (Combretastatin A4 phosphate potassium salt) (Compound 8)

cis-1-(3-O-Phosphate-4-methoxyphenyl)-2-(3,4,5-  
 35 trimethoxyphenyl)ethene ammonium salt (0.50g, nominally 1.16mmol) was dissolved in water (100ml) and treated with Dowex 50 8X K<sup>+</sup> form (30ml dry resin). The mixture was

stirred in the dark for 1 hour and then filtered.

The resin was washed with water (40ml). The water portions were combined and freeze dried to give the crude potassium salt as a white fluffy solid (0.53g). The crude product was dissolved in water (35ml) and treated with acetone (500ml). A white crystalline solid separated out which was collected by filtration and dried in vacuo to give combretastatin A4 phosphate potassium salt as a white crystalline solid (0.30g, 0.63mmol, 55% yield). mp 177.4-179.1°C (decomp. corrected).

$^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  ppm reference from  $\delta$  HOD=4.80 ppm: 3.66 (s, 6H, OMe); 3.71 (s, 3H, OMe); 3.78 (s, 3H, OMe); 6.49 (d, 1H,  $J=12.0\text{Hz}$ , ArCH); 6.62 (d, 1H,  $J=12.0\text{Hz}$ , ArCH); 6.64 (s, 2H, ArH); 6.83 (overlapping multiplets, 2H, ArH); 6.83 (broad s, 1H, ArH). IR (nujol mull)  $1560\text{cm}^{-1}$  (C=C);  $1505\text{cm}^{-1}$  (Ar);  $1460\text{cm}^{-1}$  (Ar);  $1260\text{cm}^{-1}$  (phosphate).

20

### EXAMPLE 3a

Preparation of cis-1-(3-O-(2-carbamoylethanoate)-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene ethyl ester (Combretastatin A4 glycine carbamate ethyl ester) (Compound 9).

cis-1-(3-Hydroxy-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (0.5g, 3.16mmol) was dissolved in dry tetrahydrofuran (10ml), and ethyl isocyanatoacetate (0.23g, 3.48mmol) was added, followed by triethylamine (0.20g, 3.48mmol). After stirring the solution for 12 hours at 25°C, the solvents were removed under reduced pressure to afford a white crystalline solid which was redissolved in ethyl acetate (30ml) and washed with water (2x50ml). Evaporation of the solvent under reduced

35



pressure gave combretastatin A4 glycine carbamate ethyl ester as an amorphous white powder (0.60g, 1.35mmol, 86% yield), mp 110-114°C (decomp. corrected).

5           <sup>1</sup>H NMR (DMSO-D<sub>6</sub>) δ ppm from TMS 1.17 (s, 3H, CH<sub>2</sub>CH<sub>3</sub>); 3.59 (s, 6H, OMe); 3.63 (s, 3H, OMe); 3.73 (s, 3H, OMe); 3.80 (d, 2H, CH<sub>2</sub>); (4.08, q, 2H, CH<sub>2</sub>CH<sub>3</sub>); 6.38 (1H, d, J=12.2Hz, ArCH); 6.40 (1H, d, J=12.2Hz, ArCH); 6.53 (2H, s, Ar-H); 6.70 (1H, d, J=8.3Hz, Ar-H); 6.74 (1H, 10 dd, J=8.3Hz, 2.0Hz, Ar-H); 6.84 (1H, d, J=2.0Hz, Ar-H).

### EXAMPLE 3b

15 Preparation of cis-1-(3-O-[2-carbamoylethanoate]-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (Combretastatin A4 glycine carbamate) (Compound 10).

          cis-1-(3-O-[2-Carbamoylethanoate]-4-methoxyphenyl)-  
20 2-(3,4,5-trimethoxyphenyl)ethene ethyl ester (0.6g, 1.35 mmol) was dissolved in tetrahydrofuran (10ml), and sulphuric acid (2.0M, 10ml) was added. After stirring for 12 hours at 60°C, the solution was poured into water (100ml) and the mixture was extracted with ethyl acetate  
25 (3 x 50ml). The combined extracts were washed with water (100ml), dried (Na<sub>2</sub>SO<sub>4</sub>), and the solvent was removed under reduced pressure to afford the product as a white powder (0.47g, 1.12mmol, 84% yield) mp 124-126°C (corrected).

30 <sup>1</sup>H NMR (DMSO-D<sub>6</sub>) δ ppm referenced from tetramethylsilane (TMS): 3.60 (s, 6H, OMe); 3.63 (s, 3H, OMe); 3.69 (d, 2H, CH<sub>2</sub>); 3.73 (s, 3H, OMe); 6.48 (1H, d, J=12.2Hz, ArCH); 6.51 (1H, d, J=12.2Hz, ArCH); 6.52 (2H, s, Ar-H); 6.71 (1H, d, J=8.3Hz, Ar-H); 6.74 (1H, dd, J=8.3Hz, 2.0Hz, Ar-  
35 H); 7.01 (1H, d, J=2.0Hz, Ar-H); 7.97 (1H, t, NH); 12.2-13.0 (1H, brs, COOH).

MS (EI) m/e=417 (M<sup>+</sup>).

## Elemental analysis

	Required (%)	Found (%)
C	60.43	60.35
H	5.52	5.64
5 N	3.36	3.39

EXAMPLE 4

10

Alternative Preparation of Combretastatin A4 Phosphate  
potassium salt

Another synthesis of Combretastatin A4 phosphate  
15 salt has been performed without first synthesising  
Combretastatin A4 as an intermediate. In this  
alternative method of preparation 3-hydroxy-4-methoxy-  
benzaldehyde was converted to its butyl imine,  
phosphorylated to give 4-methoxy-3-(0-phosphate)-  
20 benzaldehyde bis t-butyl ester and then coupled with  
3,4,5-trimethoxybenzyltriphenylphosphonium ylide to give  
a 3/1 mixture of Combretastatin A4 phosphate bis t-butyl  
ester and its trans-isomer. The isomer mixture was  
processed to provide the potassium salt which was  
25 recrystallised to give a 9/1 mixture of Combretastatin A4  
phosphate potassium salt and its trans-isomer. More  
complete details are set forth below.

(a) 3-Hydroxy-4-methoxybenzyl-n-butyldimine  
30 (Compound 11)

3-Hydroxy-4-methoxybenzyl-n-butyldimine (5.0g,  
32.9mmol), n-butyldimine (6ml, 60.8mmol) and p-toluene-  
sulphonic acid mono-hydrate (0.1g, 0.5mmol) were heated  
together at reflux in toluene (150ml) in conjunction with  
35 a soxhlet apparatus charged with magnesium sulphate for  
24 hours. The solvent and excess n-butyldimine were  
removed by evaporation to give crude 3-Hydroxy-4-

methoxybenzyl-n-butyldimine as a brown oil (6.1g, 29.5mmol), 90%). This was used in the next stage without purification.

<sup>1</sup>H-NMR Spectrum:

5 (CDCl<sub>3</sub>) δ ppm from TMS; 0.94 (t, 3H, J=7.3Hz, CH<sub>2</sub>CH<sub>3</sub>); 1.37 (m, 2H, CH<sub>2</sub>CH<sub>3</sub>); 1.62 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>); 3.57 (td, 2H, J=7.1, 1.1Hz, NCH<sub>2</sub>); 3.92 (s, 3H, OCH<sub>3</sub>); 6.87 (d, 1H, J=8.3Hz, ArH); 7.21 (dd, 1H, J=8.3, 2.0Hz, ArH); 7.34 (d, 1H, J=2.0Hz); 8.14 (s, 1H, NH).

10 Mass Spectrum:

CI(NH<sub>3</sub>)m/e; 208 (M+H); 137 (M-butylamine).

(b) 4-Methoxy-3-(O-phosphate)benzaldehyde bis t-butyl ester (Compound 12)

15 1-H-Tetrazole (3.4g, 48mmol) was added to a stirred solution of 3-Hydroxy-4-methoxybenzyl-n-butyldimine (5.0g, 24.0mmol) and N,N-diethyl-bis-O-t-butylphosphoramidite (7.2g, 29mmol) in THF (100ml). The mixture was stirred at room temperature for 2h and then cooled to -78°C. A  
20 solution of m-chloroperoxybenzoic acid (8g, 46mmol) in dichloromethane (100ml) was added. After 30 minutes saturated sodium thiosulphate aqueous solution (50ml) was added and the mixture was allowed to warm to room temperature. The mixture was extracted with  
25 dichloromethane (3 x 50ml). The extracts were combined and washed with sodium hydrogen carbonate solution (100ml), dried over magnesium sulphate and the solvent was evaporated under reduced pressure. The residue was chromatographed on silica, eluting with ethyl acetate-  
30 petrol (60-80) 1/1. Fractions corresponding to R<sub>f</sub> 0.20 (silica, ethyl acetate-petrol (60-80) 1/1) were combined and evaporated to give 4-Methoxy-3-(O-phosphate) benzaldehyde bis t-butyl ester as a white solid 0.7g, 2.03 mmol, 8.5% yield).

35 <sup>1</sup>H - NMR Spectrum:

(CDCl<sub>3</sub>) δ ppm from TMS; 1.55 (s, 18H, CHCH<sub>3</sub>); 3.94 (s, 3H, OCH<sub>3</sub>); 7.07 (d, 1H, Ar-H); 7.67 (d, 1H, Ar-H);

7.89 (s, 1H, Ar-H); 9.86 (s, 1H, CHO) 288.

<sup>13</sup>C - NMR Spectrum:

(CDCl<sub>3</sub>) δ ppm from TMS; 29.7 (CHCH<sub>3</sub>); 55.7 (OCH<sub>3</sub>);  
111.7 (protonated aromatic); 120.7 (protonated  
5 aromatic); 128.0 (protonated aromatic); 129.3  
(quaternary aromatic); 139.9 (quaternary aromatic);  
140.6 (d, J=6.9Hz, C-O-P); 155.6 (d, J=6.3Hz, C-O-P);  
190.9 (CN).

<sup>31</sup>P - NMR Spectrum:

10 (CDCl<sub>3</sub>) δ ppm from phosphoric acid; -15.04.

Mass Spectrum:

EI m/e=; 344 (M); 151 (M-P(O)(OC<sub>4</sub>H<sub>9</sub>)<sub>4</sub>).

(c) Cis-1-(3-O-Phosphate-4-methoxyphenyl)-2-(3,4,5-  
15 trimethoxyphenyl)ethene bis t-butyl ester  
(Combretastatin A4 phosphate bis t-butyl ester)  
(Compound 6)

n-Butyllithium (2.5M in hexanes, 0.6ml, 1.5mmol)  
20 was added to a stirred ice-cold suspension of 3,4,5-  
trimethoxybenzyl triphenylphosphonium bromide (0.76g,  
1.46mmol) in THF (30ml) under argon. The mixture was  
stirred at room temperature for 1 hour during which time  
a deep-red coloured homogeneous solution was formed. 4-  
25 Methoxy-3-(0-phosphate)benzaldehyde bis t-butyl ester  
(0.50g, 1.45mmol) dissolved in THF (5ml) was added and  
the solution was stirred in the dark for 1 hour. The  
reaction mixture was poured onto water (20ml) and  
extracted with ethyl acetate (50ml). The organic layer  
30 was dried over magnesium sulphate and the solvent was  
evaporated under reduced pressure. The residue was  
chromatographed on silica, eluting with ethyl acetate-  
petrol (60-80) 1/1. Fractions corresponding to R<sub>f</sub> 0.23  
(silica, ethyl acetate-petrol (60-80) 1/1) were combined  
35 and evaporated to give a 3/1 mixture of Combretastatin A4  
phosphate bis t-butyl ester and its trans-isomer (as  
assessed by proton NMR spectroscopy) as a colourless oil

(0.51g, 1.0mmol, 69% yield). This stilbene isomer mixture was used as such in the next step without any further purification.

5

(d) Cis-1-(3-O-Phosphate-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene potassium salt  
(Combretastatin A4 phosphate potassium salt)  
(Compound 8)

10

Trifluoromethanesulphonic acid (8 drops) was added to an ice-cold solution of the stilbene isomer mixture (0.14g, 0.28mmol) in dichloromethane (10ml) in the dark. After 1 hour the solvent was evaporated under reduced pressure. Ethanol (10ml) was added and then evaporated under reduced pressure. This was repeated twice. The residue was dissolved in ethanol (5ml). Concentrated ammonia aqueous solution ("0.88", 6 drops) was added and a white precipitate Combretastatin A4 phosphate ammonium salt formed. This was collected by filtration, dissolved in water (10ml) and stirred with Dowex (Regd. Trade Mark) 50 X8 cation exchange resin in the K<sup>+</sup> form for 0.5 hour. The resin was removed by filtration and the aqueous filtrate was freeze-dried. The white fluffy solid obtained was recrystallised from water-ethanol to give Combretastatin A4 phosphate potassium salt and its trans-isomer in a 9/1 ratio as a white crystalline solid (16mg, 0.034mmol, 12% yield). The proton NMR spectrum of the major species in the mixture corresponded exactly with the proton NMR spectrum of the Combretastatin A4 phosphate potassium salt obtained by the synthesis via Combretastatin A4 detailed earlier.

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#### Practical Usage

As already indicated, the novel analogues or derivatives of Combretastatin A4 provided by the present

invention, especially although not exclusively such analogues or derivatives which are biodegradable in vivo to Combretastatin A4 and which are soluble in water, for example Combretastatin A4 phosphate or salts thereof, amino-acid carbamate derivatives, carbohydrate derivatives and polyhydroxylated derivatives, are particularly useful as pro-drugs that may be made up into pharmaceutical formulations for administration in the therapeutic treatment for example of mammals suffering from neoplastic diseases or cancer.

In making up such pharmaceutical formulations in the form of sterile liquid preparations for parental use for instance, a predetermined therapeutically effective non-toxic amount of the particular analogue or derivative concerned may be dissolved in phosphate buffered saline and the preparations may be presented in unit dosage form and contained in sealed ampoules ready for use. In general, at least in aqueous solution, concentrations not greater than 2mg/ml will be preferred, but the amount and dosage routine required for optimum effectiveness will of course vary and is ultimately at the discretion of the medical or veterinary practitioner treating the mammal in each particular case.

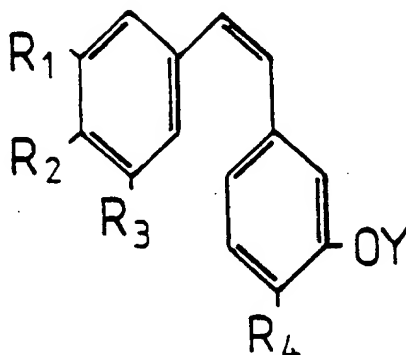
As will be seen, the invention provides a number of different aspects and, in general, it embraces all novel and inventive features and aspects, including novel compounds, herein disclosed either explicitly or implicitly and either singly or in combination with one another. Moreover, the scope of the invention is not to be construed as being limited by the illustrative examples or by the terms and expressions used herein merely in a descriptive or explanatory sense.

CLAIMS

1. A process for preparing a substituted diphenyl-ethylene compound having the general structure

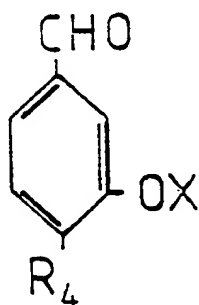
5

I  
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15 wherein  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are alkoxy groups, and Y is hydrogen, a phosphate or phosphate derivative, an amino acid carbamate derivative, or a derivative of a carbohydrate or polyhydroxylated compound, the process being characterised in that it is a convergent process  
20 that includes the step of reacting, in a Wittig coupling reaction, an ylide triphenyl phosphonium salt of a trialkoxy benzyl halide with a benzaldehyde compound:

25



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wherein X is a protective group.

2. A process as claimed in Claim 1 wherein the protective group X of the benzaldehyde compound is a  
35 silyl group.

3. A process as claimed in Claim 2 wherein the

benzaldehyde compound is 4-alkoxy-3-(thexyldimethylsilyloxy)benzaldehyde.

4. A process as claimed in Claim 3 wherein  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are each methoxy groups, further characterised in that it comprises the steps of reacting 3,4,5-trimethoxybenzyl bromide (obtainable by brominating 3,4,5-trimethoxybenzyl alcohol) with triphenylphosphine to produce the corresponding triphenylphosphonium salt, followed by reacting the triphenylphosphonium salt (in the form of its ylide) with 4-methoxy-3-(thexyldimethylsilyloxy)benzaldehyde (obtainable by silylating 3-hydroxy-4-methoxybenzaldehyde) thereby to effect said Wittig coupling and produce a mixture of silylated Combretastatin A4 and its trans isomer, separating said silylated Combretastatin A4 by chromatography, and removing the protective silyl group to produce Combretastatin A4.

5. A process as claimed in Claim 4, further characterised in that the Combretastatin A4 is phosphorylated to produce Combretastatin A4 phosphate bis *t*-butyl ester which is then hydrolysed to provide the corresponding ammonium salt, followed by isolating said ammonium salt and converting it into a corresponding alkali metal salt.

6. A process as claimed in Claim 4, further characterised in that the Combretastatin A4 is reacted with ethyl isocyanatoacetate and triethylamine to produce Combretastatin A4 glycine carbamate ethyl ester, followed by the steps of isolating said glycine carbamate ethyl ester and subsequently removing the ethyl ester group to produce Combretastatin A4 glycine carbamate.

7. A process as claimed in Claim 1 wherein the protective group X of the benzaldehyde compound is a



phosphate ester.

8. A process as claimed in Claim 7 wherein the benzaldehyde compound is a 4-alkoxy-3-(0-phosphate) benzaldehyde bis *t*-butyl ester.

9. A process as claimed in Claim 8 wherein  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are each methoxy groups, further characterised in that it comprises the steps of reacting 3,4,5-trimethoxybenzyl bromide (obtainable by brominating 3,4,5-trimethoxybenzyl alcohol) with triphenylphosphine to produce the corresponding triphenylphosphonium salt, followed by reacting the triphenylphosphonium salt (in the form of its ylide) with 4-alkoxy-3-(0-phosphate) benzaldehyde bis *t*-butyl ester (obtainable by phosphorylating a butylimine derivative of 3-hydroxy-4-methoxybenzaldehyde) thereby to effect said Wittig coupling and produce a mixture of Combretastatin A4 phosphate bis *t*-butyl ester and its *trans* isomer, followed by further processing to remove the *t*-butyl ester groups and to form the corresponding ammonium salt of the isomers, then isolating said ammonium salt and converting it into a corresponding alkali metal salt.

10. A water soluble Combretastatin A4 analogue or derivative which has a structure as defined in Claim 1 wherein Y is other than hydrogen and which is obtainable by a process as claimed in any of the preceding claims.

11. A compound as claimed in Claim 10 which is one of the following:

- (1) *cis*-1-(3-O-Phosphate-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene ammonium salt;
- (2) *cis*-1-(3-O-Phosphate-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene potassium salt.

12. A Combretastatin A4 analogue or derivative obtainable by a process as claimed in any of Claims 1 to 9 which is one of the following:

- 5 (1) *cis*-1-(3-0-(2-carbamoylethanoate)-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene ethyl ester;
- (2) *cis*-1-(3-0-[2-carbamoylethanoate]-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene.

10 13. A compound useful as an intermediate in the synthesis of Combretastatin or of an analogue or derivative thereof, said compound being selected from 4-Methoxy-3-(thexyldimethylsilyloxy)benzaldehyde, *cis*-1-(4-Methoxy-3-(thexyldimethylsilyloxy)phenyl)-2-(3,4,5-trimethoxyphenyl)ethene and 4-Methoxy-3-(O-phosphate)-benzaldehyde bis *t*-butyl ester.

15

14. A pharmaceutical composition or formulation for medical use comprising a therapeutically useful and effective non-toxic amount of a compound having the structure specified in Claim 1 and made by a process as claimed in any of the preceding claims, together with a pharmaceutically acceptable carrier, diluent or excipient.

20

25 15. A pharmaceutical composition or formulation as claimed in Claim 14 wherein said compound is selected from *cis*-1-(3-0-Phosphate-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene ammonium salt, *cis*-1-(3-0-Phosphate-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene potassium salt, *cis*-1-(3-0-(2-carbamoylethanoate)-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene ethyl ester and *cis*-1-(3-0-[2-carbamoylethanoate]-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene.

30

35 16. A pharmaceutical composition or formulation for medical use comprising a therapeutically useful and

effective non-toxic amount of a compound as claimed in Claim 10, together with a pharmaceutically acceptable carrier, diluent or excipient.

5 17. A pharmaceutical composition or formulation as claimed in Claim 16 wherein said compound is Combretastatin A4 phosphate or a salt thereof.

10 18. Use of a compound as claimed in any of Claims 10 to 12 for the manufacture of a medical preparation or medicament for the treatment of neoplastic disease in humans or other animals.

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 92/00498

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.C1.5      C 07 C 43/23      A 61 K 31/66      A 61 K 31/27 C 07 F 9/12      C 07 F 7/18      C 07 C 271/54		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
Int.C1.5	C 07 C 43/00      C 07 C 271/00      C 07 F 7/00 C 07 F 9/00      A 61 K 31/00	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>		
Category <sup>o</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X	EP,A,0276051 (ARIZONA STATE UNIVERSITY) 27 July 1988, see claims; examples 12,36 <div style="text-align: center;">---</div>	1-4,13
A	Experientia, vol. 45, 1989, Birkhäuser Verlag, CH-4010, (Basel, CH), G.R. PETTIT et al.: "Isolation and structure of the strong cell growth and tubulin inhibitor combretastatin A-4", pages 209-211, see the whole document (cited in the application) <div style="text-align: center;">-----</div>	1-18
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>o</sup> Special categories of cited documents : <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
12-06-1992	05.08.92	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	Wright	

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB92/00498

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☒ Claims Nos.: 1-3,7,8,10,14,16,18  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
  
The expressions "a derivative of a carbohydrate or polyhydroxylated compound" and "protective group" in claim 1 do not permit a full search.  
The search has been based on the examples.
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
  
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

GB 9200498  
SA 57564

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0276051	27-07-88	DE-A- 3864269	26-09-91
		JP-A- 63233939	29-09-88
		US-A- 4996237	26-02-91
-----			

**EPO FORM P0479**

BNSDOCID: <WO\_\_\_9216486A1\_I\_>